Optimization and Evaluation of an FFPE Dual Extraction Protocol for Next-Generation Sequencing Applications

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Background
Formalin-fixed paraffin-embedded (FFPE) biopsies are highly valuable and widely used tissue specimens for clinical diagnostics. However, obtaining sufficient and high-quality nucleic acid material from limited FFPE samples presents a challenge for downstream molecular analysis, such as next-generation sequencing (NGS). We present an optimized sequential extraction method that generates high-quality DNA and RNA from a single set of input tissues that is automatable and operation-friendly.

Methods
8 FFPE samples were macrodissected and nucleic acid were extracted by using 4 different extraction kits. DNA and RNA yield, quality, purity and impacts on NGS assay performances were evaluated.

Overview of Extraction Workflow

Fig 1A: The general workflow of each kit. The Promega kit does start with a separate DNA and RNA process that ends on the Maxwell. The other 3 kits follow a sequential workflow allowing DNA to be separated from the RNA-containing supernatant after a Proteinase K incubation time and processed independently. Omega and ThermoFisher both utilize the KingFisher.

Fig 1B: Clinical FFPE samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Tumor Content (%)</th>
<th>Tumor Size (mm)</th>
<th>Tissue Type</th>
<th>Tissue Thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>07</td>
<td>80%</td>
<td>27</td>
<td>Endometrium</td>
<td>1 section; 80 µm</td>
</tr>
<tr>
<td>08</td>
<td>50%</td>
<td>15</td>
<td>Lung</td>
<td>1 section; 80 µm</td>
</tr>
<tr>
<td>10</td>
<td>70%</td>
<td>16</td>
<td>Colon</td>
<td>1 section; 80 µm</td>
</tr>
<tr>
<td>11</td>
<td>30%</td>
<td>25</td>
<td>Ovary and Oviduct, Left</td>
<td>1 section; 80 µm</td>
</tr>
<tr>
<td>12*</td>
<td>70%</td>
<td>16</td>
<td>Collectomy</td>
<td>1 section; 80 µm</td>
</tr>
<tr>
<td>13</td>
<td>40%</td>
<td>28</td>
<td>Colon</td>
<td>1 section; 80 µm</td>
</tr>
<tr>
<td>14</td>
<td>30%</td>
<td>23</td>
<td>Ascitic Fluid (Abdomen)</td>
<td>1 section; 80 µm</td>
</tr>
<tr>
<td>15</td>
<td>70%</td>
<td>16</td>
<td>Lymph node, Right Flank</td>
<td>1 section; 80 µm</td>
</tr>
</tbody>
</table>

DNA yield (ng/µl) by Kit

Fig 2A: Omega has the highest DNA yields across samples and good consistency.

RNA Yield (ng/µL) by Kit

Fig 2B: Omega and Covaris have comparable RNA yields.

DNA DV200 by Kit

Fig 2C: Promega had slightly longer RNA fragments

Fig 2D: DV200 is comparable between Omega and Covaris kit

DNA Yield

RNA Yield

TSO500HT Feasibility Data

Fig 3: Omega manual DNA extraction at 40 ng input had higher library complexity than Promega

Comparison between Omega and Covaris kit

Fig 5A: Omega kit showed there is no significant differences with using mineral oil versus no mineral oil treatment prior to the proteinase K digestion on deparaffinized tissue samples.

Deparaffinized Slides

Fig 5B: Omega kit showed there is no significant differences with using mineral oil versus no mineral oil treatment prior to the proteinase K digestion on deparaffinized tissue samples.

Key findings
An optimized sequential extraction method generates high-quality DNA and RNA from a single set of input tissues that is automatable and operation-friendly. This workflow performs well with reduced FFPE tissue input and efficiently supports various high-throughput clinical NGS applications.

Conclusions
An optimized FFPE extraction method allows more clinical biopsy samples to be tested with different NGS workflows, providing a better diagnostic value for patient care.